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Na^+/H^+ exchange and its inhibition in cardiac ischemia and reperfusion

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Summary: The characterization of various ion transport systems has led to a better understanding of the effects, which seem to take part in the impairment of ischemic and reperfused cardiac tissue. This review discusses the role of the Na^+/H^+ exchange system in the pathophysiology of ischemia and reperfusion and the beneficial effects of its inhibition.

At the onset of ischemia intracellular pH (pH_i) decreases due to anaerobic metabolism and ATP hydrolysis, leading to an activation of Na^+/H^+ exchange. This in turn increases intracellular Na^+ (Na^+_i) and activates Na^+/K^+ ATPase, with a consecutive increase of energy consumption. Since cellular Na^+ and Ca^{++} transport are coupled by the Na^+/Ca^{++} exchange system, which depends on the Na^+ gradient, the high Na^+_i leads to increased intracellular Ca^{++} (Ca^{++}_i). After a certain period, Na^+/H^+ exchange is inactivated by a decrease of extracellular pH.

In case of reperfusion the acid extracellular fluid is washed out, which reactivates Na^+/H^+ exchange, leading to an unfavourably fast restoration of pH_i and a second time to Na^+ and Ca^{++}_i overflow.

High Ca^{++}_i is assumed to be one of the main reasons for ischemic and reperfusion injury, like arrhythmias, myocardial contracture, stunning and necrosis.

It seems that the inhibition of Na^+/H^+ exchange can interrupt this process at an early phase and prevent or delay the consequences of ischemia and reperfusion as demonstrated by numerous investigators.

Key words: Na^+/H^+ exchange inhibition – cardiac ischemia – reperfusion – intracellular pH – intracellular Na^+ – intracellular Ca^{++}

Introduction

In the last decade it has become clear, that most pathophysiological processes in cardiac ischemia and reperfusion are connected to a derangement of cellular ion homeostasis. The characterization of various ion transport systems has led to the identification of three effects, which seem to play key roles in the impairment of ischemic and reperfused tissue. These interdependent effects are decreased intracellular pH (pH_i), intracellular Na^+ (Na^+_i) overload and intracellular Ca^{++} (Ca^{++}_i) overload.

The interdependence between intracellular acidosis and elevated Na^+_i and Ca^{++}_i concentrations is based on the activity of certain ion transport systems. Increased activity of the Na^+ coupled HCO_3^- transport and an excessive activation of Na^+/H^+ exchange by a decrease of pH_i lead to a significant elevation of Na^+ influx into the endangered tissue (50, 70, 76, 97). As long as sufficient ATP is available, the intruding Na^+ ions can be transported against the Na^+ gradient into the extracellular space by the Na^+/K^+ ATPase (25, 75). But eventually, decreasing energy stores and elevated Na^+ influx result in a marked increase of the concentration of Na^+_i (25, 46). Since intracellular Na^+ and Ca^{++} concentrations are linked by a $3Na^+/Ca^{++}$ exchange (7, 41, 55), the elevation of Na^+_i finally causes intracellular Ca^{++} overload (26, 30, 58, 97), which induces cardiac arrhythmias (14, 90) and necrosis (90).

Already in 1984 it has been suggested, that Na^+/H^+ exchange could play an important role in this chain of pathophysiological events (25). While the regulation of pH_i might be dominated by HCO_3^- dependent systems under normal conditions (99), it has been claimed that the exchanger causes a major part of the Na^+ influx in cardiac ischemia and reperfusion (25, 46, 58, 94, 97). More recently, this point of view has been supported by studies showing a significant reduction of Na^+ and Ca^{++} overload (2, 39, 58, 111) and impressive cardioprotective effects with inhibitors of the Na^+/H^+ exchanger (16, 33, 38, 79, 84, 85). Altogether this points to a crucial involvement of this ion transport system in the mechanisms of ischemic and reperfusion injuries.

In the following, this review tries to summarize and interpret the current data on the role of Na^+/H^+ exchange in the pathophysiology of cardiac ischemia and reperfusion. It will then discuss the effects of an inhibition of the exchanger under these conditions.

Role of Na^+/H^+ exchange in ischemia

It seems that there are at least three phases of cardiac ischemia. The first phase would be the time until the ischemic myocardium ceases to contract, the second phase would be the period of ischemic arrhythmias and the third phase would be characterized by a lack of mechanical and electrical activity.

At the beginning of an ischemic insult myocardial cells are subjected to numerous changes in metabolism and ion transport activity. Since the remaining oxygen in the tissue is diminished within few seconds (73) the physiological way of energy production by degrading fatty acids in the mitochondria (104) is inhibited and the production of energy depends on anaerobic glycolysis (1, 92).

However, energy consumption will continue, as for example for the maintenance of ion gradients by Na^+/K^+ ATPase (71). This degradation of ATP is accompanied by a production of protons (17) most of which would be neutralized by the metabolic activity inside the mitochondria under physiological conditions (17). Since mitochondrial activity ceases after few seconds of ischemia (81, 107) the accumulation of protons leads to a decrease of pH_i (59, 61).

The ischemic cells counteract this process by an activation of several pH regulating cellular ion transport systems like lactate transport (100), Na^+ dependent HCO_3^- transport (99) and Na^+/H^+ exchange (25, 73).

There is a controversy about the dominance of the different pH_i regulation systems in ischemia. However, several investigators found that recovery from low pH_i depends on Na^+/H^+ exchange (99, 106) and that the exchanger seems to be the dominant pH-regulating system in acidified cardiac cells (25, 62). Furthermore, it has been shown in acidified cells (25) as well as in isolated hearts that Na^+/H^+ exchange accounts for the majority of Na^+ influx during pH recovery in ischemia and reperfusion (58, 94, 97). Since the activity of the Na^+/K^+ pump is controlled by the concentration of Na^+_i (28), the increased Na^+ influx is followed by an activation of the ATP consuming ion pump. However, degradation of ATP will generate more protons (17) and it will also enhance anaerobic glycolysis (36) and lactate formation. This process creates a vicious cycle that will lead to a further activation of the Na^+ dependent pH regulating systems and to a further increase in Na^+_i.

Even in these first seconds of ischemia degradation of ATP, anaerobic glycolysis, decrease of pH_i, and Na^+ influx are very likely interconnected by several feed-back loops. Decrease of pH_i is caused by anaerobic metabolism and degradation of ATP (17). This drop of pH_i itself causes enhanced Na^+ influx, increased ATP consumption (99, 106), more anaerobic metabolism and consequently a further drop of pH_i. On the other hand, ATP degradation and anaerobic glycolysis as well as numerous intracellular processes depend on pH_i and are

reduced by intracellular acidosis (77). Thus, the cycle of ATP consumption and proton generation can run unhampered only as long as protons can be extruded efficiently to the extracellular space and as long as substantial amounts of ATP are available.

A fast onset of the drop of pH_i and a decrease of ATP of about 50 % within 10 minutes has been observed in ischemic rat hearts at 37 °C (23, 27, 58). Within the same time pH_i reached a value of about 6.3.

What are the possible reasons for the observed decrease in pH_i and ATP? Protons, which are generated by the anaerobic metabolism, are transported into the extracellular space. Since they are not washed out during ischemia, the extracellular pH can be expected to decrease. In fact, this has been demonstrated by several investigators (12, 13, 83), and it has been shown that extracellular pH (pH_o) decreases to about 6.0 in ischemic myocardial tissue within 15–20 minutes (23, 24, 27, 42, 57). In consequence, this accumulation of protons in the extracellular space will increasingly inhibit the extrusion of more protons from the ischemic cells (101, 102). In addition, Na^+-coupled HCO_3^- transport will function only as long as HCO_3^- is available in the extracellular environment, and lactic acid transport will be hampered by a decreasing gradient for lactate and protons across the cell membrane. Consequently, a decrease of pH_i seems inevitable relatively soon after the onset of ischemia.

ATP decreases because anaerobic glycolysis is a much less efficient way of energy production than normal cellular metabolic pathways, yielding only five per cent of the regular amount. In addition, anaerobic glycolysis depends on intracellular glycogen stores, which are depleted during ischemia, because of lacking glucose supply in the ischemic tissue. Another reason is the fall of pH_i itself, which inhibits anaerobic glycolysis like most other enzyme-driven pH dependent cellular reactions (60, 77, 78).

There are several consequences of decreased pH_i and ATP. One consequence is a decrease of myocardial contractility, which is observed very soon after the beginning of ischemia (11, 31, 52, 53, 63, 66, 91, 92, 103). Since ATP is required for the contractile process, a reduction of myocardial contraction can be expected with ATP deficiency. However, the reported drop of ATP of only about 10–15 % (10, 29, 109) in this first phase of ischemia does not well correlate with the fast reduction of myocardial contraction. In contrast, the faster decrease of pH_i (13, 20, 57) gives a much better correlation. This is in agreement with the diminished binding of Ca^{++} to the contractile elements in acidified myocardial cells (8, 22, 53) as well as the reduced activity of the myosin-ATPase at low pH (8, 40, 80). In addition, myocardial contractility seems to be very sensitive to only small reductions of pH_i (57).

As reported earlier, pH_i regulating ion transport systems cause Na^+ influx (25, 46, 71, 100). However, a decrease of intracellular ATP will reduce the capacity of the ATP dependent Na^+/K^+ ATPase to eliminate Na^+ from the intracellular space. In fact, a marked increase of Na^+_i in ischemic cardiac tissue has been observed by numerous investigators (48, 50, 58, 70, 76, 97). In parallel with the increase of Na^+ in ischemic cardiac tissue an increase of intracellular Ca^{++} has been found (58,94). The Ca^{++} levels in myocardial cells are significantly regulated by $3Na^+/Ca^{++}$ exchange (7, 41, 55). This exchanger is driven by the electrical potential of the cell and by the Na^+ and Ca^{++} gradient (7, 41, 55) and it extrudes Ca^{++} under physiological conditions (7, 41, 55). The transport rate of the $3Na^+/Ca^{++}$ exchange is diminished by intracellular Na^+ overload (26, 30, 46, 47, 69, 74, 97) and the exchanger might even change the direction of its transport if Na^+_i becomes excessively high (25, 46, 98).

High Ca^{++}_i levels are thought to be deleterious to ischemic myocardium in several ways (14, 26, 90). Together with low ATP high Ca^{++}_i has been connected with overexcitability and electrical instability (4). In general, it is well documented that high Ca^{++}_i is one of the main reasons for ischemic arrhythmias (and reperfusion arrhythmias) (65).

Cardiac arrhythmias usually do not start immediately at the beginning of an ischemic insult. In patients undergoing Percutaneous Transluminal Coronary Angioplasty (PTCA) very few arrhythmias are observed during the one or two minutes of cardiac ischemia, which the patients have to suffer (64). In anesthetized rats with experimental ligation of the left coronary artery marked arrhythmic activity starts after 5 minutes of ischemia and continues for about 10 more minutes (84, 85). These events might reflect a certain degree of cellular Na^+ and Ca^{++} overload after 5 minutes of ischemia. Indeed, NMR measurements in rat hearts show significant increases of Na^+_i (35, 70) and Ca^{++}_i (23) after this period of ischemia.

It may also be interesting to consider the pathophysiological process in adrenergic nerve endings in the heart during ischemia and reperfusion. One of the significant activities of these cells is the uptake of norepinephrine from the extracellular space by a Na^+ driven uptake mechanism ($uptake_1$) (87, 96). Inside the cells norepinephrine is transported into storage vesicles in exchange for protons (6, 68, 108).

During an ischemic insult adrenergic nerve endings very likely undergo a similar derangement of metabolism and ion homeostasis as myocardial cells (88). There should also be a decrease of pH_i and an increase of Na^+ influx through Na^+/H^+ exchange, leading to an intracellular Na^+ overload and a decrease of ATP (3, 87, 88). It has been shown that the activity of the Na^+-dependent norepinephrine $uptake_1$ is diminished by high Na^+_i and that it might also change its direction of transport (87).

Furthermore, the storage mechanism of norepinephrine in the vesicles depends on energy. It will become insufficient when ATP decreases in ischemia (6, 68, 108), leading to intracellular norepinephrine overflow (6, 68, 108). A high intracellular norepinephrine concentration together with high Na^+ will cause an extracellular overflow of norepinephrine through reversed transport of the $uptake_1$ (87, 96).Under such conditions the extracellular norepinephrine concentration in cardiac tissue might rise a hundred fold above normal (87). Since the arrhythmogenic potential of high norepinephrine concentrations is well known (15, 49), norepinephrine overflow might be one of the arrhythmogenic factors in ischemic cardiac tissue (88).

Arrhythmias in cardiac ischemia cease after 10 to 15 min in the rat model, although Na^+ and Ca^{++} overload continue to increase beyond that time (58, 94). Furthermore, pH_i decreases down to about 6.0 after 20–30 min (23, 24, 27, 35, 42, 57), which is accompanied by a pronounced decrease of ATP in the same time (23, 27, 35, 94). In this respect, it is remarkable that neither pH_i nor pH_o tend to drop below a value of about 6.0, even if ischemia lasts longer than 15–30 min (23, 35).

This lack of further acidification could indicate a stop of a significant metabolism in the ischemic myocardial cells. Anaerobic glycolysis, which is known to depend on pH_i (60, 77, 78), is very likely inhibited when pH_i reaches values as low as 6.0. Without energy the maintenance of ion gradients, necessary for the electrical potentials of the cells, is impossible. Therefore, ischemic cells become electrically quiescent (43) and arrhythmic activity will stop in the ischemic core area.

Although intracellular Ca^{++} overload (58, 94) cannot induce further arrhythmic activity it will, however, destroy mitochondria (9, 89, 90), activate lysosomes (45), destabilize the cell membrane (32, 90), and finally cause cellular necrosis (90). Without reperfusion this might be the inevitable fate of the endangered cells.

Role of Na^+/H^+ exchange in reperfusion

Reperfusion, for the rescue of the ischemic tissue, is a double-edged sword. In the beginning it causes a very rapid washout of the acidic extracellular fluid in the ischemic area.

This generates a huge pH-gradient between intracellular and extracellular space. In consequence, the pH-regulating cellular ion transport systems are reactivated to a maximum degree, which will cause a large Na^+ influx by Na^+/H^+ exchange (46) and Na^+ dependent HCO_3^- transport (100).

The Na^+ ions cannot be extruded sufficiently by the Na^+/K^+ ATPase due to a lack of ATP, leading to an additional intracellular Na^+ overload (97) and, secondary, to an additional $Ca^{++}{}_i$ overload (93) by $3Na^+/Ca^{++}$ exchange (58, 95). This process is paralleled by a very fast restoration of pH_i (12, 23, 100). While elevated $Ca^{++}{}_i$ in myocardial cells is less efficacious at low pH_i (8, 22, 53) it will increasingly take effect when normal pH_i is restored. The results are reperfusion arrhythmias (65), reperfusion injuries like myocardial contracture (5, 94), myocardial stunning (5, 42, 94), and necrosis (5, 72, 94).

Energy depleted local sympathetic nerve endings face the same process as ischemic myocardium during reperfusion (87). In the nerve endings Na^+ overload and ATP depletion again result in norepinephrine overflow by the above described mechanism (87). This perpetuates arrhythmias (15, 67) and enhances Ca^{++} influx (104) in the destabilized myocardial tissue.

Adverse effects of reperfusion seem to be limited to an early phase. Reperfusion experiments with acidic solutions, which keep Na^+/H^+ exchange and Na^+ coupled HCO_3^- transport inactivated, have shown, that this vulnerable phase in cardiac reperfusion might last for about two minutes (4). While two minutes of acidic reperfusion had a maximum protective effect in cardiac tissue, reperfusion, continued with physiological pH after two minutes, had no further adverse effects (4). This finding suggests, that after two minutes of reperfusion without further Na^+ overflow and a delayed restoration of pH_i, intracellular ATP is restored so far that the reperfused cells might cope with enhanced Na^+ influx and might reduce elevated $Ca^{++}{}_i$.

In summary, Na^+/H^+ exchange seems to play a key role in the pathophysiology of ischemia and reperfusion in multiple ways, and beneficial effects from Na^+/H^+ exchange inhibition in cardiac ischemia and reperfusion can be expected.

Inhibition of Na^+/H^+ exchange in ischemia

In ischemia cardiac cells rapidly shift to anaerobic metabolism followed by activation of Na^+/H^+ exchange (73). Therefore, one of the first significant effects of Na^+/H^+ exchange inhibition in ischemia should be a reduction of cellular Na^+ influx (46). This might have several consequences (Figs. 1a and 1b). Primarily, there would be less Na^+ ions to be extruded by the Na^+/K^+ pump. Secondarily, the ATP consumption by the Na^+/K^+ pump would be reduced and $Na^+{}_i$ overload would be prevented or at least delayed. The next consequence would be a reduction of the following intracellular Ca^{++} overload via the $3Na^+/Ca^{++}$ exchange (54). Furthermore, a prevention of Ca^{++} overload should prevent ischemic arrhythmias if ischemic arrhythmias result from increased $Ca^{++}{}_i$.

Most of these conclusions are already supported by experimental evidence. In fact, a marked reduction of $Na^+{}_i$ overload and a conservation of ATP (84, 85) by Na^+/H^+ exchange inhibition in cardiac ischemia has been observed by several investigators (2, 51, 111). In addition, it seems that Na^+/H^+ exchange inhibition causes a diminution of $Ca^{++}{}_i$ overload (2, 39, 58, 111). Furthermore, ischemic arrhythmias can almost completely be prevented by Na^+/H^+ exchange inhibitors (79, 84, 85).

Since the exchanger has a significant role in the regulation of pH_i in cardiac ischemia (99, 100), the decrease of pH in ischemic myocardial cells should be accelerated by Na^+/H^+ exchange inhibition. This would enhance the inhibition of anaerobic glycolysis (60, 77, 78) and reduce the degradation of ATP (77). In addition, less protons would be generated by

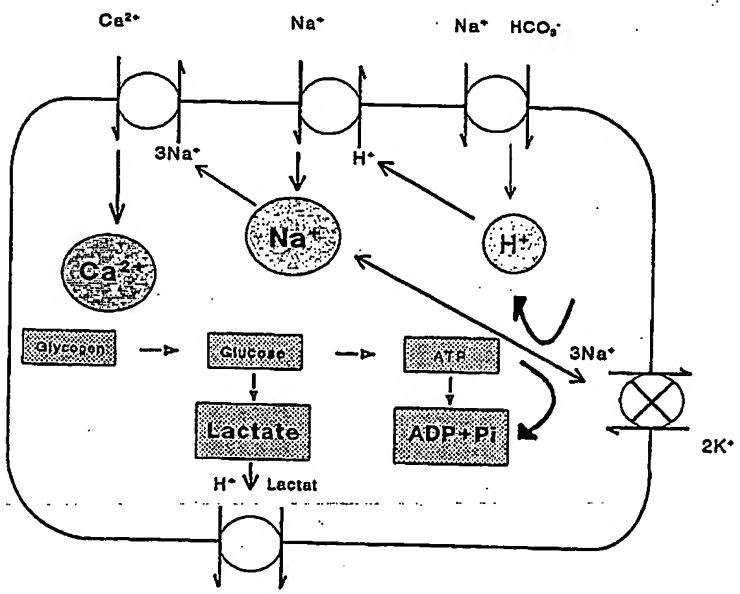

Fig. 1a. Active Na^+/H^+ exchange in ischemic cardiac myocytes: Elevated intracellular Na^+ concentrations cause an intracellular Ca^{++} overload. Anaerobic metabolism is driven by high intracellular Na^+ and the excretion of H^+.

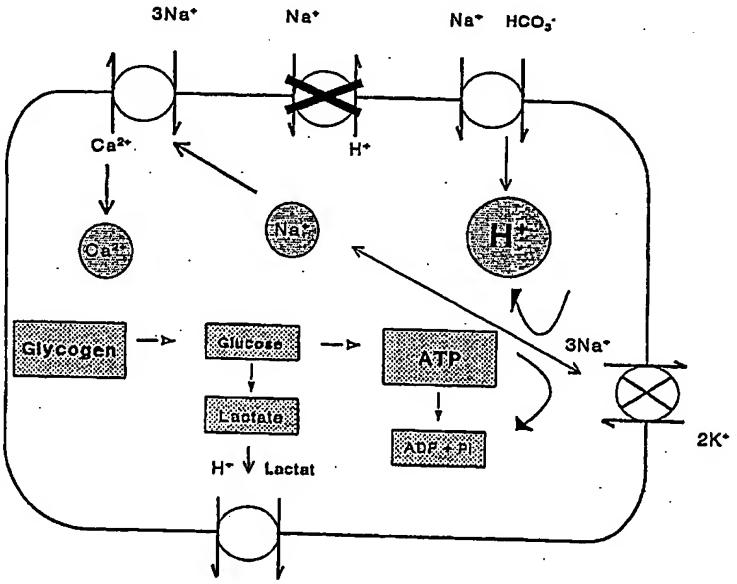

Fig. 1b. Inhibited Na^+/H^+ exchange in ischemic cardiac myocytes: Reduced intracellular Na^+ concentrations prevent intracellular Ca^{++} overload. Anaerobic metabolism is inhibited by reduced intracellular Na^+ and intracellular acidification.

ATP break down, since a decreased Na^+ influx would reduce ATP consumption by Na^+/K^+ATPase.

At present, there is only few experimental evidence for an influence of an inhibition of the exchanger on the pH_i decrease in ischemia. While the decrease of pH_i seemed slightly faster

in ischemic ferret hearts pre-treated with the Na^+/H^+ exchange inhibitor EIPA (100), another investigator did not find any effect of amiloride on the rate of fall of pH_i in rat hearts (58). However, the shortcoming of most NMR studies using the phosphate shift to investigate changes of pH_i in ischemia and reperfusion is the relatively infrequent data acquisition of pH like every 5 or 10 min. Thus, an early difference in pH_i by Na^+/H^+ exchange inhibition would not necessarily be recognized.

On the other hand, Na^+/H^+ exchange inhibition causes a diminution of lactate efflux from ischemic cardiac tissue as well as a decrease of cellular lactate content (84, 85). These observations suggest a decrease of lactate formation, which would implicate a reduction of anaerobic glycolysis. In the same studies these effects were paralleled by a conservation of intracellular glycogen stores (84, 85). Since glucose has to come from intracellular glycogen stores during ischemia, this observation also indicates a reduction of anaerobic glycolysis by Na^+/H^+ exchange inhibition.

A faster decrease of pH_i by Na^+/H^+ exchange inhibition in ischemia could have further beneficial effects. One of them could be a reduced activity of the $3Na^+/Ca^{++}$ exchange which has been found to depend on pH_i (21, 37). Another beneficial effect might be a reduction of free radical formation, which is also depressed by low pH_i (110). A third one might be an earlier onset of the decrease of contractility of ischemic myocardial tissue. This has been shown with EIPA (83) and might result in a conservation of cellular energy stores. However, there are only few experimental data available yet on these possible effects of Na^+/H^+ exchange inhibition.

A conservation of cellular energy stores, as observed with Na^+/H^+ exchange inhibition in myocardial tissue might also prevent or diminish norepinephrine overflow in ischemia from intracellular vesicles in the local sympathetic nerve endings (88). Furthermore, a reduced Na^+_i overload (88) might avert the release of norepinephrine by a reversed $uptake_1$ mechanism (88).

In fact, such a prevention of norepinephrine overflow in ischemic cardiac tissue has been demonstrated in several experiments with different Na^+/H^+ exchange inhibitors (87). These effects in neuronal tissue in the heart might contribute to the reduction of Ca^{++}_i overload and arrhythmic activity in cardiac ischemia, which has been observed with Na^+/H^+ exchange inhibitors.

As mentioned above, in prolonged ischemia, when arrhythmic activity has finally ceased, a continuing Ca^{++}_i overload will cause irreversible injury and finally necrosis of the ischemic cardiac tissue (90) by damaging mitochondria (9, 89, 90), activating lysosomes (45) and destabilizing cellular membranes (32, 90).

Na^+/H^+ exchange inhibitors might prevent or delay this process by a marked reduction of Ca^{++}_i in ischemic cardiac tissue (58). This assumption has been partially confirmed by studies using isolated ischemic working rat hearts. In these experiments Na^+/H^+ exchange inhibitors markedly decreased the release of the intracellular enzymes creatine kinase and lactate dehydrogenase, indicating a reduction of cellular membrane instability and necrosis (84, 85).

It is hard to predict, how long the protection by Na^+/H^+ exchange inhibition could last. In human hearts "hibernating myocardium" has been identified with positron emission tomography after relatively long periods of reduced flow (82). Although there is not much known about the processes involved, this observation demonstrates that a protection of underperfused cardiac tissue for days or even weeks might be possible. Nevertheless, the best chance of the ischemic myocardium for survival and restoration of contractile function is reperfusion. The benefits of reperfusion might be markedly increased, if reperfusion injury could be diminished.

Inhibition of Na^+/H^+ exchange in reperfusion

As described above, the sudden removal of the acidic extracellular fluid during reperfusion leads to a second excessive Na^+ influx (4). Since both Na^+ influx and the fast rise in pH_i are mostly caused by Na^+/H^+ exchange, an inhibition of the exchanger in the vulnerable phase of reperfusion might significantly reduce reperfusion injuries.

A decrease of Na^+ influx during reperfusion would again reduce ATP consumption by the Na^+/K^+ATPase (46) and would prevent Na^+_i and Ca^{++}_i overflow (25, 46). In parallel, a retarded recovery of pH_i would protect the reperfused cardiac tissue from the effects of the high Ca^{++}_i concentrations (8, 22, 40, 53, 80) gained during ischemia (93).

Ischemia-reperfusion experiments with Na^+/H^+ exchange inhibitors have already demonstrated some beneficial effects like a marked reduction of intracellular Na^+ and Ca^{++} overload in reperfused myocardium (83), a slow down of pH_i recovery (100), increased ATP levels (84, 85), and a prevention of norepinephrine overflow (87).

In consequence, all these actions of Na^+/H^+ exchange inhibition in reperfusion seem to have a common outcome, which is the observed prevention of intracellular Ca^{++} overload (58). Since excessively elevated intracellular Ca^{++} is thought to be the reason for adverse effects of reperfusion, like arrhythmias (14, 26, 90), myocardial contracture (5, 94), myocardial stunning (5, 94), and necrosis (5, 72, 94), a prevention of these effects by Na^+/H^+ exchange inhibition could be expected. In fact, a diminution of reperfusion arrhythmias with Na^+/H^+ exchange inhibitors has been demonstrated recently in vitro and in vivo by numerous investigators (16, 38, 79, 83–85). In parallel, a prevention of the increase of left ventricular enddiastolic pressure (18, 33, 58), a prevention of myocardial stunning in isolated hearts (33) and in anesthetized pigs (79), and a reduction of myocardial damage (38) have been observed.

Besides a reduction of intracellular Na^+ and Ca^{++} overload in reperfused cardiac tissue (58), Na^+/H^+ exchange inhibitors could be even more protective if they are also present during the ischemic period. Cardiac tissue might be in a better condition then to meet reperfusion stress. At the time being, only few studies have been reported in which Na^+/H^+ exchange inhibitors were employed during both, ischemia and reperfusion, or during reperfusion only (38). However, recent experiments with blood perfused rabbit hearts, using the highly specific Na^+/H^+ exchange inhibitor HOE 694 (84), clearly demonstrated a better prevention of myocardial contracture and a better recovery of contractility when the inhibitor was present during ischemia and reperfusion (33).

Inhibition of Na^+/H^+ exchange in low flow ischemia, in hypoxia and in reperfusion after low flow ischemia

Experiments under so-called "low flow ischemia" gave controversial results about the involvement of the Na^+/H^+ exchanger. This variance probably derives from different degrees of ischemia reached in the different experiments.

While in one study, with isolated nonworking rat hearts, under low flow conditions only a small decrease of pH_i and ATP occurred, and the Na^+/H^+ exchange inhibitor EIPA had no effect on intracellular ATP or pH (34), another study showed a marked decrease of intracellular pH under hypoxic conditions (105). Other investigators have found a decrease of intracellular Na^+ and Ca^{++} overload by Na^+/H^+ exchange inhibition (2).

Two more studies reported significant protection by amiloride derivatives against reperfusion injury after low flow ischemia (16, 18).

Conclusion

While the part of Na^+/H^+ exchange in the regulation of pH_i might be negligible under normal conditions (99), the exchanger is markedly activated in ischemia and reperfusion (25,

46, 71, 100). Furthermore, evidence has been accumulated since 1985 for a multiple involvement of Na^+/H^+ exchange in the pathophysiology of cardiac ischemia and reperfusion.

Several studies have demonstrated that the inhibition of the exchanger in cardiac ischemia and reperfusion definitely decreases Na^+_i and Ca^{++}_i overload (2, 39, 58, 83, 111). In addition, inhibitors of Na^+/H^+ exchange cause a reduction of anaerobic glycolysis (84, 85) and diminish ATP depletion (51, 84–86).

As expected, these effects were paralleled by a reduction of arrhythmias and necrosis in cardiac ischemia (19, 56), as well as by a prevention of reperfusion injury (18, 33, 38, 58, 79, 85). These observations fit elegantly to the assumed significant role of the exchanger in the pathophysiology of ischemia and reperfusion. However, their conclusiveness depends on the specificity of the actions of the Na^+/H^+ exchange inhibitors used on the exchange system.

While amiloride, as the first substance found to affect Na^+/H^+ exchange, has qualified as a relatively weak and non-specific inhibitor (44), several potent and rather selective amiloride derivatives, like EIPA, DMA, MIBA, HMA and others have been identified in the last decade (44). In addition, a new and highly specific compound, HOE 694, which has been characterized as rather potent and selective inhibitor of the exchanger, has been employed in several studies (33, 79, 84). The protective effects, which all these compounds have shown in cardiac ischemia and reperfusion, are comparable in their principle quality. Therefore, these effects seem to be definitely related to Na^+/H^+ exchange inhibition.

In summary, pathophysiological evidence and experimental observations have qualified Na^+/H^+ exchange inhibition as a possible new therapeutic principle in cardiac ischemia and reperfusion.

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